Volume 182, number 2 FEBS 2364 March 1985

Protein S4 is near the elongation factor G binding site in the ribosome

F. Tejedor and J.P.G. Ballesta*

Centro de Biología Molecular, CSIC and UAM, Canto Blanco, Madrid-28049, Spain

Received 18 January 1985

Using a mild iodination method for protein radioactive labeling, it has been shown that elongation factor G, when bound to the ribosome as EFG-GDP-fusidic acid complex, protects protein S4 from labeling. The results indicate that protein S4 is probably near the ribosomal EFG binding site.

Ribosomal protein *Elongation factor G* *Iodination*

1. INTRODUCTION

The elongation factor G (EFG), interacting with the ribosomes after the formation of the peptide bond, allows the translocation of the peptidyl-tRNA from site A to site P during protein synthesis. The binding site for EFG is, therefore, an important part of the ribosome structure which, in addition, probably overlaps with the interaction site for the elongation factor T_u [1]. The identification of the ribosomal components involved in this active region is of particular interest in order to understand the elongation factors' mode of action.

Early studies suggested the involvement of the acidic proteins L7 and L12 in the interaction of the elongation factors with the ribosome [2,3]. Afterwards, different types of experiments showed that, although these proteins are important for the elongation factors' activity, they do not form part of the actual binding site [4,5].

Affinity labeling studies identified proteins L5, L11 and L18 as involved in the EFG-dependent GTPase center, which must be close to the EFG binding site [6], and more recently cross-linking experiments indicated that the factor interacts at the interface between ribosomal subunits since proteins from both particles can be cross-linked to the factor [7].

* To whom correspondence should be addressed

From all the data available, obtained from cross-linking, affinity labeling and electron microscopy studies [6–9], a crude but descriptive picture of the elongation factor interaction site is emerging. However, more data, obtained if possible by different techniques, are required for a complete description of this important ribosomal region. We have identified protein S4 as probably involved in EFG interaction by using a differential iodination technique.

2. MATERIALS AND METHODS

Ribosomes were prepared from *Escherichia coli* MRE 600 cells grown in YEP medium and ground with alumina following standard methods [4]. 70 S ribosome-EFG-GDP-fusidic acid complexes were isolated as described by Bodley et al. [10]. In this case, GTP and fusidic acid, both at 10^{-4} M, were always present in the buffers during preparation and storage of the particles.

Iodination of ribosomes was carried out following either the standard chloramine T method or the modified system (two phase system) that keeps chloramine T separate from the ribosomes, as described elsewhere [11]. In both cases, 100–150 μg of particles in 50 μl of 50 mM phosphate (pH 7.4), 10 mM $MgCl_2$ were labeled with 50–100 μCi of $Na^{125}I$. Chloramine T was at 1 mg/ml in the standard method.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Ribosomal proteins, extracted from the particles with 66% acetic acid and precipitated from the extract with 5 vols acetone [12], were separated either by standard 2-dimensional gel electrophoresis [13] or by monodimensional electrophoresis in the presence of SDS.

To extract proteins from gels after SDS electrophoresis, the stained spots were cut and washed 20 times × 4 h with 1 ml of 7% acetic acid in 25% ethanol to remove the SDS and afterwards treated with 50% acetic acid as described elsewhere [14] to release the proteins. The extracted proteins, after lyophilization, were dissolved in a sample buffer for 2-dimensional gel electrophoresis.

3. RESULTS

We recently described a method for iodination of biological samples, called the two phase system, which by avoiding direct contact of chloramine T with the substrate minimizes chemical damage to the material and labels preferentially the surface of the biological structures [11].

The characteristics of the two phase system induced us to use this labeling method as a tool to detect changes in the ribosome structure due to the interaction of different effectors.

When ribosomes are prepared in the presence of fusidic acid, the interaction of the elongation factor G with the particle is stabilized and 70 S-EFG-GDP complexes are obtained [10]. Iodination of these complexes by the two phase system results in a protein labeling pattern clearly different from the iodination pattern obtained in free 70 S ribosomes (fig.1). As expected, a prominent radioactive band in the elongation factor region of the gels is detected only in protein extracted from 70 S-EFG complexes, but in addition, a band corresponding to a ribosomal protein of 25–30 kDa is present in the free 70 S ribosomes and absent in the EFG-carrying particles. These data suggest that the binding of the elongation factor G to the ribosome protects this ribosomal protein from labeling.

On the other hand, our results confirm the specificity of the iodination method used, since when the experiments are carried out using the standard chloramine T technique, no difference in the labeling of the 25–30 kDa protein is detected (not shown).

Similar experiments performed with purified

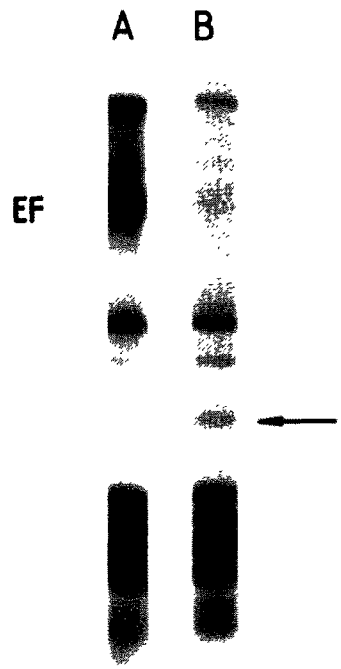

Fig.1. Autoradiogram of SDS-gel electrophoresis of proteins from: (A) 70 S-EFG-fusidic acid complexes iodinated with the 'two phase' system. (B) 70 purified ribosomes iodinated with the two phase system. EF indicates the region where the elongation factor moves in the stained gel. The arrow points to the position of the protected protein.

EFG and 70 S ribosomes did not give such clear-cut results, probably due to the large fraction of ribosomes that do not form the complex (about 43%) together with the low stability of the complex formed under these conditions [15].

In order to identify the protected protein, the radioactive band, extracted from the SDS gels of labelled 70 S ribosomal proteins, was rerun, with unlabelled 70 S proteins as carrier, in a standard 2-dimensional electrophoresis. As shown in fig.2, the radioactive spot in the autoradiogram of the gel coincides with the position of protein S4 on the Coomassie blue-stained plate.

4. DISCUSSION

The results presented in this communication show that protein S4 is protected from iodination by the EFG-GDP-fusidic acid complex bound to the 70 S ribosome. These results can only be obtained when a very mild iodination method that preferentially labels the external surface of the particle [11] is used. The standard iodination technique using chloramine T, is too drastic and either releases the EFG from the ribosome or, more probably, is also able to label the internal components of the particle.

Other authors have also used external radioactive labeling as a way to detect conformational alterations in the ribosomal structure caused by different effectors [16–19]. These alterations have been inferred from results showing relative changes in the level of protein iodination that in some cases only correspond to 10–20% of the labeling. Accepting these data as significant, our results, showing a total lack of labeling of S4, together with the strong iodination of the factor bound to the particle, suggest that the effect caused by the binding of the elongation factor to this protein is not due to a conformational effect but rather to direct physical protection.

Protein S4 has been reported, among others, as close to the EFG binding site by photochemical cross-linking studies [7]. Girshovich et al. [9] have shown that protein S12 can also be directly cross-linked to EFG. Since S4 is a neighbour of S12 [20] our data are in agreement with these reports and confirm an implication of S4 in the EFG ribosomal binding site.

ACKNOWLEDGEMENTS

This work was supported by institutional grants to the Centro de Biología Molecular from Fondo de Investigaciones Sanitarias (Instituto Nacional de Previsión) and by personal grants to J.P.G.B. from Comisión Asesora de Investigación Científica. We thank Ma. Carmen Fernández Moyano for expert technical assistance.

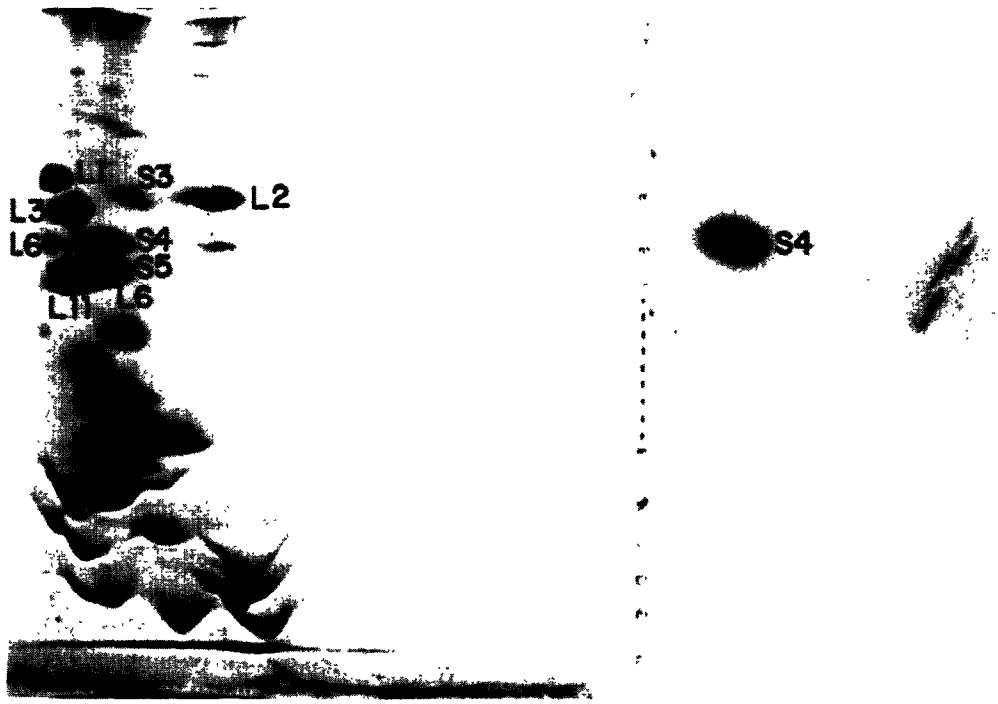

Fig.2. Two-dimensional gel electrophoresis of iodinated protein extracted from SDS-gels using unlabeled 70 S proteins as carrier. (A, left) Stained gel; (B, right) autoradiogram.

REFERENCES

[1] Ballesta, J.P.G. and Vázquez, D. (1972) in: Ribosomes: Structure, Function and Biogenesis (Bloemendal, H. et al. eds) North Holland/American Elsevier, Amsterdam.

[2] Möller, W. (1974) in: Ribosomes (Nomura, M. et al. eds) pp.711–731, Cold Spring Harbor Laboratory, New York.

[3] Acharya, A.S., Moore, P.B. and Richards, F.M. (1973) Biochemistry 12, 3108–3114.

[4] Ballesta, J.P.G. and Vázquez, D. (1972) FEBS Lett. 28, 337–342.

[5] Hamel, E., Koka, M. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814.

[6] Maassen, J.A. and Möller, W. (1974) Proc. Natl. Acad. Sci. USA 71, 1277–1280.

[7] Maassen, J.A. and Möller, W. (1981) Eur. J. Biochem. 115, 279–285.

[8] Girshovich, A.S., Kurtskhalia, T.V., Ovchinnikov, Y.A. and Vasiliev, V.D. (1981) FEBS Lett. 130, 54–59.

[9] Girshovich, A.S., Bochkareva, E.S. and Ovchinnikov, Y.A. (1981) J. Mol. Biol. 151, 229–243.

[10] Rohrbach, M.S., Dempsey, M.G. and Bodley, J.W. (1974) J. Biol. Chem. 249, 5094–5101.

[11] Tejedor, F. and Ballesta, J.P.G. (1982) Anal. Biochem. 127, 143–149.

[12] Barritault, D., Expert-Benzanzon, A., Guerin, M.F. and Hayes, D. (1976) Eur. J. Biochem. 63, 131–135.

[13] Howard, G.A. and Traut, R.R. (1974) Methods Enzymol. 30, 526–539.

[14] Bernabeu, C., Conde, F.P. and Vázquez, D. (1978) Anal. Biochem. 84, 97–102.

[15] Campuzano, S., Vázquez, D. and Modolell, J. (1979) Biochemistry 18, 1570–1574.

[16] Litman, D.J. and Cantor, C.H. (1974) Biochemistry 13, 512–518.

[17] Michalski, C.J. and Sells, B.H. (1974) Eur. J. Biochem. 49, 361–367.

[18] Martinez, O., Vázquez, D. and Modolell, J. (1978) FEBS Lett. 87, 21–25.

[19] Martínez, O. and Modolell, J. (1979) Mol. Biol. Rep. 5, 193–196.

[20] Sommer, A. and Traut, R.R. (1975) J. Mol. Biol. 97, 471–481.